Membrane-bound NAD(P)-independent malate dehydrogenase (EC 1.1.99.16) was purified to homogeneity from the membrane of thermotolerant *Acetobacter* sp. SKU 14, an isolate from Thailand. The enzyme was solubilized from the membrane fraction of glycerol-grown cells with 1% Triton X-100 in the presence of 0.1 M KC1, and purified to homogeneity through steps of column chromatographies on DEAE-Sephadex A-50 and DEAE-Toyopearl in the presence of 0.1% Triton X-100. The purified enzyme showed a single protein band in both native-PAGE and SDS-PAGE. The enzyme was a homodimer with a molecular mass of 60 kDa subunit and had noncovalently bound FAD as the cofactor. The enzyme was stable over pH 5 and had its maximum activity at pH 11.0 when ferricyanide was used as an electron acceptor. The enzyme activity was elevated by the addition of ammonium ions. The substrate specificity was very strict to only L-malate, of which the apparent $K_m$ was 10 mM and over 20 compounds involving D-malate were not oxidized by the enzyme.